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Serum selenium status of HIV-infected children on care and treatment in Enugu, Nigeria

A C Ubesie,[1,2] MBBS, MPH, FMCPaed, FWCAP; **B C Ibe**,[1,2] MBBS, FMCPaed, FWCAP; **I J Emodi**,[1,2] MBBS, FMCPaed, FWCAP; **K K Iloh**,[2] MBBS, FMCPaed, FWCAP

[1] *Department of Paediatrics, College of Medicine, University of Nigeria, Nsukka, Nigeria*
[2] *Department of Paediatrics, College of Medicine, University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu, Nigeria*

Corresponding author: *A C Ubesie (agozie.ubesie@unn.edu.ng)*

Objective. To compare the selenium status of HIV-infected and HIV-uninfected children.
Methods. This was a hospital-based comparative study using a structured questionnaire in the quantitative research domain at the University of Nigeria Teaching Hospital, Ituku/Ozalla, Enugu, Nigeria. Seventy-four HIV-infected children were compared with 74 non-HIV-infected children (35 males and 39 females in each group). The outcome measure was the selenium status of the study participants.
Results. The mean (standard deviation (SD)) weight-for-height *z*-score among the subjects was –0.18 (1.53) compared with 0.05 (1.68) among the controls (p=0.457). The mean (SD) height-for-age *z*-score among the subjects was –1.16 (1.44) compared with 0.06 (1.06) among the controls (p<0.001). Eighteen subjects (24.3%) compared with eight controls (11.4%) were selenium deficient (odds ratio 2.49; 95% confidence interval 1.00 - 6.18; p=0.044). Median CD4 counts of selenium-deficient and non-deficient subjects were 765.5 (range 409 - 1 489) and 694.0 (range 85 - 2 196) cells/μL, respectively (p=0.321). The proportions of selenium deficiency were 26.4% and 22.2% in the highly active antiretroviral therapy (HAART) and pre-HAART groups, respectively (p=0.565).
Conclusion. There was a significant difference in the proportion of HIV-infected children who were selenium deficient compared with their uninfected counterparts.

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Worldwide, an estimated 2.5 million children under 15 years of age are living with HIV, and more than 2.3 million of them live in sub-Saharan Africa.[1] Infections and malnutrition have been shown to be associated with increased HIV mortality.[2] HIV-related malnutrition involves both micro- and macronutrient deficiencies.[3] There is a compelling association of micronutrient deficiencies in HIV-infection with immune deficiency, rapid disease progression and mortality.[4] Micronutrient supplements can delay HIV disease progression and reduce mortality in HIV-infected persons not receiving highly-active antiretroviral therapy (HAART).[5]

Although the use of HAART has revolutionised the management of HIV infection, micronutrient deficiencies still occur among HIV-infected patients on HAART.[6] The provision of simple, inexpensive micronutrient supplements as an adjunct to HAART may therefore have several cellular and clinical benefits, such as a reduction in mitochondrial toxicity and oxidative stress and an improvement in immune reconstitution.[5] One such micronutrient is selenium, an essential trace element with antioxidant properties.[7] In humans and animals, selenium increases immune function and is required for growth and reproduction.[8] It also exerts antiviral activities by inhibiting reverse transcriptase enzyme in RNA-virus-infected animals.[9,10] Supplemental selenium can potentially prevent the replication of HIV and retard the development of AIDS in newly infected subjects.[9] Ensuring selenium sufficiency among HIV-infected children, especially in settings with a high burden of malnutrition, may improve survival. However, studies that have evaluated the prevalence of selenium deficiency among HIV-infected children are limited. This study set out to evaluate the prevalence of selenium deficiency among HIV-infected children compared with uninfected controls at the University of Nigeria Teaching Hospital (UNTH), Enugu State.

Study area and design

This was a hospital-based comparative study of selenium in HIV-infected and HIV-uninfected children carried out between October 2013 and August 2014 at the UNTH. The hospital serves as a referral centre to primary and secondary healthcare facilities within and outside south-eastern Nigeria. It is among the first generation of tertiary hospital facilities in the country. HIV-infected children are managed at the paediatric HIV clinic, which runs once a week.

Study population

The subjects were HIV-infected children aged 6 - 180 months (15 years) enrolled between October 2013 and February 2014. The control group was non-HIV-infected children, matched for age, sex and socioeconomic status, who were recruited from the children's outpatient clinic of the teaching hospital. Socioeconomic index scores were assigned to the occupations and educational attainments of the parents or caregivers of subjects and controls using the Oyedeji socioeconomic classification scheme, which grades subjects from I to V.[11] The socioeconomic classification for each study participant was obtained by finding the mean score of his or her parents. If either of the parents was dead, the score of the surviving parent was used, and if both parents were dead, the score of the caregiver was used. Classes I and II were regarded as upper social class, III as middle and IV and V as lower social class.

Inclusion criteria

The subjects were confirmed HIV-infected children aged 6 - 180 months (15 years), and the controls were HIV-uninfected children on follow-up visits at the children's outpatient clinic matched for age, sex and socioeconomic status with the subjects.

Exclusion criteria

Children aged ≥7 years who refused to assent to the study or whose caregivers refused consent, as well as those with a history of micronutrient supplementation in the past 3 months, were excluded.

Consent

Thumb-printed and/or signed informed consent was obtained from the parents or caregivers, while assent was obtained from study participants aged ≥7 years.

Recruitment of study participants

Children who met the inclusion criteria were enrolled consecutively until the desired sample size for the subjects and controls was achieved. Seventy-four HIV-infected children served as the subjects, while non-HIV-infected children matched for age, sex and socioeconomic status served as the controls. A structured questionnaire was used to collect the following data from the subjects and controls: age in months, date of birth, date of interview, and the highest educational attainment and occupation of parents or caregivers. Data on HAART regimen and duration of treatment were retrieved from the medical records of the HIV-infected children. The controls were screened for the presence of HIV antibodies using the national algorithm for HIV testing.

The study participants were examined for the presence of any clinical signs of illness. An infant weighing scale (Hospibrand ZT-120, UK) was used to measure the weights of children under 2 years to the nearest 0.1 kg, while a standing scale was used for children aged 2 years and above. Weight measuring instruments were set to zero point before use and standardised at weekly intervals using known weights. Length was measured using an infantometer (Seca, Germany) for children under 2 years while height was measured for children 2 years and above using a stadiometer to the nearest 0.1 cm.

Nutritional assessment

Height-for-age *z*-score (HAZ), weight-for-height *z*-score (WHZ) and body mass index-for-age *z*-score (BMIZ) values were calculated using the 2005 World Health Organization (WHO) AnthroPlus version 1.0.4 software calculator (Switzerland).[12] Acute malnutrition (wasting) was defined as WHZ and BMI *z*-scores ≤–2 while chronic malnutrition (stunting) was defined as HAZ ≤–2.[13]

Laboratory tests

Two aliquots of 5 mL of blood were collected from the antecubital fossa of the subjects. The first aliquot for CD4 estimation was collected in an ethylenediaminetetraacetic acid (EDTA) bottle. The second aliquot for selenium was collected in plain bottles. Similarly, a 5 mL aliquot of blood was collected in plain bottles from the antecubital fossa of controls for selenium estimations. The blood samples for CD4 estimation were analysed using the Partec CyFlow machine (Germany). Serum selenium was measured by the spectrophotometric method using the Safranin O method. The following are recommended normal selenium levels:[14,15]

- <18 months: 30 - 50 µg/L (0.38 - 0.63 µmol/L)
- 18 months - 4 years: 45 - 90 µg/L (0.57 - 1.14 µmol/L)
- 5 - 16 years: 55 - 115 µg/L (0.70 - 1.46 µmol/L)
- adults (>16 years): 70 - 130 µg/L (0.89 - 1.65 µmol).

In this study, therefore, selenium deficiency was defined as follows:

- <18 months: <30 µg/L (0.38 µmol/L)
- 18 months - 4 years: <45 µg/L (0.57 µmol/L)
- 5 - 15 years: <55 µg/L (0.70 µmol/L).

Data analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 19.0 (IBM Corp., USA). The χ^2 and Fisher's exact tests were used to test for the significant association of categorical variables. Fisher's exact test was used if the expected number in a cell of a two-by-two table was less than five, and Yates' correction if a cell contained zero. The quantitative data were tested for normality using the Shapiro-Wilk normality test. A Student *t*-test was used to compare the mean WAZ and HAZ between the subjects and controls. Mann-Whitney *U*- and Kruskal-Wallis tests were used to test for a significant association between CD4 count/selenium levels and independent variables. The odds ratio (ORs) of selenium deficiency between subjects and controls was calculated, and 95% confidence interval (CIs) reported. All analyses were done at the 5% level of significance and $p<0.05$ was considered statistically significant.

Ethical approval

The hospital's Health Research and Ethics Committee approved the study (ref. no. NHREC/05/01/2008B-FWA00002458-IRB00002323).

Results

Study participants

One hundred and forty-eight participants (74 subjects, 74 controls) were included in the study. The sociodemographic characteristics of the study population are shown in Table 1. A blood sample for selenium analysis was available in 74 subjects and 70 controls. The median ages of the subjects and controls were 94.8 and 84.0 months, respectively (range 7 - 180 months). Forty (54.1%) of the subjects and 37 (50.0%) of the controls were from the middle social class (p=0.777).

CD4 counts and antiretroviral (ARV) regimen

The median CD4 count of the subjects was 741.5 (interquartile range (IQR) 472.0 - 1

Table 1. Sociodemographic characteristics of the study population

Sociodemographic characteristics	Subjects, *n* (%)	Controls, *n* (%)	χ^2	df	*p*-value
Age group (years)					
0.5 - 5.0	25 (33.8)	24 (32.4)	0.147	2	0.923
5.1 - 10.0	32 (43.2)	31 (41.9)			
10.1 - 15.0	17 (23.0)	19 (25.7)			
Sex					
Male	35 (47.3)	35 (47.3)	0.0	1	1.0
Female	39 (52.7)	39 (52.7)			
Socioeconomic class					
Upper	10 (13.5)	13 (17.6)	0.508	2	0.777
Middle	40 (54.1)	37 (50.0)			
Lower	24 (32.4)	24 (32.4)			

Table 2. Comparison of the mean anthropometric parameters of the subjects and controls

Anthropometric parameters	Subjects, mean (SD)	Controls, mean (SD)	*t*-value	*p*-value
Weight (kg)	24.3 (9.6)	26.1 (13.8)	–0.91	0.364
Height (cm)	118.4 (21.6)	121.5 (25.0)	–0.80	0.426
WHZ	0.2 (1.5)	–0.1 (1.7)	0.718	0.457
HAZ	–1.2 (1.4)	0.1 (1.1)	–5.677	<0.001
BMIZ	–1.5 (15.3)	–0.2 (1.9)	–0.706	0.481

202.8 cells/μL. Fifty-six of the 74 subjects (75.7%) were on HAART, while 10 (17.9%) of the 56 children on HAART had been switched to a second-line regimen. Zidovudine (AZT), Lamivudine (3TC) and Nevirapine (NVP) were the first-line combination therapy in 46 (82.1%) of the 56 children on HAART.

Nutritional status

The mean (standard deviation (SD)) weight and height of the subjects and controls were 24.3 (9.5) v. 26.1 (13.8) kg, and 118.4 (21.6) v. 121.5 (25.0) cm, respectively (Table 2). The mean WHZ among the subjects was –0.2 (1.5) compared with –0.1 (1.7) among the controls (p=0.457). The mean HAZ among the subjects were –1.2 (1.4) compared with 0.1 (1.1) among the controls (p<0.001) (Table 2).

Five (6.8%) of the 74 subjects and 5 (6.8%) of the 74 controls had acute malnutrition (Fisher's exact test, p=1). Conversely, 18 (24.3%) subjects compared with none of the controls had chronic malnutrition (p<0.001).

Serum selenium levels of study participants

The mean (SD) serum level of selenium among the subjects was 82.1 (6.3) μg/L compared with 97.3 (7.1) μg/L among the controls (p=0.11). The median serum selenium levels for subjects and controls were 76.9 (IQR 50.8 - 98.1) μg/L and 83.30 (IQR 61.6 - 113.8) μg/L (p=0.085).

Selenium deficiency

Eighteen (24.3%) of the 74 subjects compared with eight (11.4%) of 70 controls were selenium deficient (p=0.044). The OR of selenium deficiency among the subjects was 2.49 times higher among the subjects than the controls (OR 2.49; 95% CI 1.00 - 6.18; z=1.9; p=0.049).

Age and serum selenium levels

The median ages of selenium-deficient and non-deficient study participants were 92.4 (IQR 62.4 - 123.6) and 90 (IQR 49.2 - 124.8) months, respectively (p=0.728). Seven (28.0%) of 25 subjects aged 6 - 60 months were selenium deficient (p=0.863). Six (20.7%) of 29 children aged 61 - 120 months were selenium deficient among the controls (p=0.093).

Sex and serum selenium levels

Twelve (34.3%) of 35 males compared with 6 (15.4%) of 39 females were selenium deficient among the subjects (p=0.058). Six (18.7%) of 32 males compared with 2 (5.3%) of 38 females were selenium-deficient among the controls (p=0.077) (Table 3).

Table 3. Distribution of selenium-deficient study participants by sex

	HIV-infected (n=74)*		HIV-uninfected (n=70)†	
	Yes (n %)	No (n %)	Yes (n %)	No (n %)
Sex				
Male	12 (34.3)	23 (65.7)	6 (18.7)	26 (81.3)
Female	6 (15.4)	33 (84.6)	2 (5.3)	36 (94.7)
Total	18 (22.5)	52 (77.5)	8 (11.4)	62 (88.6)

*(χ^2=3.58, df=1, p=0.058)

†(χ^2=3.12, df=1, p=0.077)

Risk factors for selenium deficiency

Nutritional status

One (20%) of the five acutely malnourished subjects was selenium deficient, compared with 17 (24.6%) of 69 without acute malnutrition (Fisher's exact test, p=1.0). The mean WHZ and BMIZ among the selenium-deficient subjects were 0.2 (0.8) and –6.9 (31.0), respectively, compared with –0.3 (1.4) and 0.2 (1.3) among non-deficient subjects. Four (22.2%) of the 18 subjects with chronic malnutrition compared with 14 (25%) of 56 subjects without chronic malnutrition were selenium deficient (Fisher's exact test, p=1). The mean HAZ among selenium-deficient subjects was –1.06 (1.61), compared with –1.09 (1.36) among the non-selenium-deficient subjects (p=0.943).

CD4 and HAART

The median CD4 counts of the 18 selenium-deficient and 56 non-deficient subjects were 765.5 (range 409 - 1 489) and 694.0 (range 85 - 2 196) cells/μL of blood, respectively (p=0.321). Fourteen (26.4%) of 53 subjects on HAART compared with four (22.2%) of 21 ART-naive subjects and eight (11.4%) of 70 controls were selenium deficient (p=0.086). There was no statistically significant difference between HAART and pre-HAART subjects who were selenium deficient (p=0.565). The mean (SD) duration of HAART for selenium-deficient subjects was 41.1 (30.8) months, compared with 43.4 (29.9) months among selenium-sufficient subjects (p=0.807).

Discussion

This study showed that a significantly higher proportion of HIV-infected children compared with their non-infected counterparts were selenium deficient. The OR of selenium deficiency was 2.5 times higher among the subjects than the controls. Lower serum selenium levels among HIV-infected indivi duals have been linked to excessive utilisation of selenoproteins by the virus.[16] It is this increased utilisation of the selenoproteins in HIV-infected individuals that results in selenium depletion. A study in Ife, south-west Nigeria, also reported a significantly higher rate of selenium deficiency among HIV-infected children compared with the uninfected controls who were matched for age and sex with the subjects.[17] However, the reported rates of selenium deficiency among the subjects and controls were higher than the findings in the present study. The group in the Ife, Nigeria, study included only ARV-naive subjects, and this may explain their reported higher proportion of selenium deficiency among HIV-infected children. The present study included both ARV-naive subjects and subjects on HAART. It is possible that HAART would have slowed down viral replications, reduced the need for selenoprotein synthesis and ultimately lowered the proportion of selenium-deficient subjects.

In contrast to the present study, Henderson *et al.*[18] reported lower selenium deficiency rates among 38 HIV-infected and -uninfected children in the USA. The lower rates found by Henderson *et al.*[18] can be explained by their small sample size of 38 subjects (28 HIV-infected and 10 HIV-uninfected), which limits the generalisability of their findings. The present study had a larger sample size of 148. Additionally, Henderson *et al.* [18] conducted their study in a country where it has been shown that adequate amounts of selenium are consumed, with an average daily intake from foods for those aged ≥2 years reaching 108.5 μg. Bunupuradah *et al.*[20] reported no deficiency in baseline selenium levels among 141 HIV-infected Thai children aged 1 - 12 years. The reason for their reported zero prevalence can be explained by their very low cut-off definition for selenium deficiency (<0.1 μmol/L or 8 μg/L) in these children.

Age and sex

The difference in the proportion of selenium deficiency among the three age groups of subjects in this study was not statistically significant. This agrees with the findings of Kouna *et al.*[21] in their study of 318 children aged 7 - 10 years. A possible explanation for this lack of significant difference may be that a wide variety of foods are rich in selenium, including seafoods, organ meats, grains and dairy products, which are consumed across age groups.[17,24] The findings of the north-east Thailand study by Krittaphol *et al.*,[22] however, disagree with those of the present

study. The authors reported that children under 9 years of age had a significantly lower mean serum selenium concentration than those over 9 years of age. The basis for dichotomising the children into under and over 9 by the authors was rather arbitrary and unclear.

Among the subjects and controls, the proportion of selenium deficiency was not significantly different between the sexes. There are conflicting reports in the literature regarding selenium levels and sex. Jones *et al.*[23] and Kouna *et al.*[21] reported no significant difference in the proportion of selenium deficiency between the sexes, which finding agrees with the present study. Rousseau *et al.*,[24] however, reported significantly lower selenium in males than females among 30 HIV-infected individuals. Safaralizadeh[25] also reported that the mean serum selenium levels were significantly lower in male than female children aged 1 - 16 years. Studies reporting lower selenium among males have failed to offer plausible explanations for the disparity. In contrast, Amare *et al.*[26] reported a higher rate of selenium deficiency in females than males. Krittaphol *et al.*[22] also reported that females had a significantly lower mean serum selenium concentration than males in their sample. Studies reporting lower selenium values among females have postulated a sex-linked hormonal influence onthe serum level of selenium.[25,26]

Nutritional status

There was no significant difference in the proportion of subjects with acute and chronic malnutrition who were selenium deficient. Nhien *et al.*[27] reported no significant difference in serum concentration of selenium with regard to underweight, stunting and wasting. Amare *et al.*[28] also reported no significant correlation between the levels of selenium and the anthropometric variables of schoolchildren. These findings suggest that selenium status in children is independent of their macronutrient status. This finding is not surprising because micronutrient deficiencies, or hidden hunger, remain a public health challenge among apparently healthy children, especially in developing countries.

Immunological status and HAART

There was no significant difference in the median CD4 count of selenium-deficient and non-deficient subjects in this study. Although Anyabolu *et al.*[17] in the Ife study reported higher mean serum selenium levels in subjects with a CD4 count of ≥350 cells/μL compared with those with <350 cells/μL , they failed to explain the rationale for grouping these children based on the CD4 counts of ≥350 and <350 cells/μL. The difference in the approach to statistical analysis of CD4 and serum selenium between their study and the present study makes it difficult to draw a meaningful comparison between the two studies.

The median serum selenium level among subjects on HAART did not differ significantly from that of those who were yet to commence HAART. There was also no significant difference in the proportion of subjects on HAART who were selenium deficient compared with the pre-ART subjects in this study. Similarly, when the proportion of selenium deficiency in the three groups of HAART, pre-HAART and controls was compared, no significant difference was found. To the best of the authors' knowledge, this is the first study that compares selenium status between HIV-infected children on HAART and those who are not. Akinola *et al.*[6] in a study involving HIV-infected adults reported no significant difference in the serum levels of selenium of their subjects on HAART and those who were yet to commence HAART, which agrees with the finding of the present study.

Study limitations

The study did not assess dietary intakes of selenium among the study participants. The exclusion of study participants based on a history of micronutrient supplements was inadequate, since there could have been recall bias.

Conclusion

Although selenium deficiency was significantly higher among the subjects than controls, no significant difference was noted between the sexes. Nutritional status, CD4 cell count and use of HAART were not significantly associated with selenium levels in this study.

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